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| <b>(54) Title:</b> USE OF GENISTEIN AND RELATED COMPOUNDS TO TREAT CERTAIN SEX HORMONE RELATED CONDITIONS<br><br><b>(57) Abstract</b><br><br>A method is disclosed for modulating the growth of tissues and cells. The method comprises contacting a cell or tissue with genistein, or with other isoflavonoids or derivatives thereof, which may be optionally coupled to a moiety which targets a particular cell or tissue. The disclosed method is particularly useful in the treatment of prostate cancer, benign prostate hypertrophy, breast cancer, and androgenic alopecia.   |           |  |

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## USE OF GENISTEIN AND RELATED COMPOUNDS TO TREAT CERTAIN SEX HORMONE RELATED CONDITIONS

### Technical Field

5           The invention relates to the modulation of the growth of tissues and cells using isoflavonoid type compounds. More specifically, the invention relates to the ability of certain isoflavonoid type compounds to treat or prevent prostate cancer, benign prostatic hypertrophy, breast cancer and androgenic alopecia.

### 10           Background Art

          There are approximately 200,000 new diagnoses each of prostate cancer and breast cancer per year in the United States. In contrast, the per capita incidence of these cancers is dramatically lower in the Japan and China. However, studies have shown that the incidences of breast and prostate cancers in first and second generation  
15       Asian immigrants to the United States approach the incidences of these cancers among Caucasians in the United States. These findings indicate that environmental factors may be involved in the etiologies of breast cancer and prostate cancer.

          A number of dietary factors have been implicated as environmental influences. First, it appears that high-fat diets correlate with the incidences of prostate cancer and  
20       breast cancer. Second, since the Asian diet is relatively high in soy bean derived products, components of soy bean have been implicated as cancer preventatives. Barnes, S. *J Nutrition* (1995) 125:777-783 have summarized studies of the relationship of soy-derived substances and cancer. Of particular interest is the study of Sharma, O.P. *et al. J Steroid Biochem Mol Biol* (1992) 43:557-564 who found that  
25       a high soy-containing diet reduced severity and incidence of prostatitis in rats. Makela, S. *et al. Institute of Technology Effects of Food on the Immune and Hormonal Systems*, Schwarzenbach, Switzerland (1991) pp. 135-139 show that a diet containing soy can prevent development of dysplastic changes in prostate of neonatally DES-treated mice. Messina, M.J. *et al. Nutr Cancer* (1994) 21:113-131  
30       cites a personal communication from M. Pollard which demonstrated that the use of a

semisynthetic diet lacking soy decreased the latency period for spontaneous tumor development in the prostate.

In particular, certain isoflavonoids isolated from soy have been studied.

5 Messina, M.J. *et al. Nutr Cancer* (1994) 21:113-131 provides an excellent review of the effects of isoflavonoids or soy containing diets on various cancers. Studies have shown enhanced isoflavonoid levels in the plasma and urine of Japanese men as compared to Finnish men. Aldercreutz, H. *et al. Lancet* (1993) 342:1209, Aldercreutz, H. *et al. Proc Ann Meet Am Assoc Cancer Res* (1995) 36:687. In addition, substantial differences in the half-lives of various isoflavonoids derived from soy have been found  
10 in men as compared to women. Lu, L.J. *et al. Proc Ann Meet Am Assoc Cancer Res* (1995) 36:A3546.

In view of these correlations, the effect of various isoflavonoids isolated from soy on prostate cancer cell line growth has been studied. Naik, H.R. *et al. Ann Anticancer Res* (1994) 14:2617-2619 showed genistein, a tyrosine kinase inhibitor  
15 purified from soy, to be cytotoxic to the rat prostate cancer cell line MAT-LyLu and the human prostate adenocarcinoma cell line PC3. Rokhlin, O.W. *et al. Cancer Lett* (1995) 98:103-110 showed that the growth of various prostatic cancer cell lines, including DU145 and LnCAP, was inhibited by various protein kinase and phosphatase inhibitors, including genistein. However, the degree of growth inhibition  
20 was greatly dependent on the specific cell line chosen and varied among the various compounds tested. In another study, Peterson, G. *et al. Prostate* (1993) 22:335-345 showed that genistein and biochanin A, but not daidzein, inhibit both serum- and EGF-stimulated growth of LnCAP and DU145 cells.

There are conflicting reports on the ability of genistein to inhibit the growth of  
25 subcutaneously implanted prostate tumor cell lines. Naik *et al.* showed that genistein failed to inhibit the growth of subcutaneously implanted MAT-LyLu cells. In contrast, Joseph, I.B. *et al. Proc Ann Meet Am Assoc Cancer Res* (1995) 37:A429 showed that a number of compounds, including genistein inhibited tumor growth of implanted MAT-Lu tumor cells. The effects of genistein were not as dramatic as  
30 those observed with linomide, however, and the authors suggested a combination

therapy of linomide with TNP-470, an angiogenesis inhibitor, as a protocol for prostate cancer.

The mechanism by which genistein might inhibit the growth of prostate tumor cell lines is unknown. One hypothesis is that genistein interferes with growth factor mediated signal transduction pathways through the inhibition of tyrosine kinases.

Sun, S.Y. *et al. Proc Ann Meet Am Assoc Cancer Res* (1995) 36:A3821 reported that various isoflavonoids isolated from soy, including genistein and daidzein, increased glucuronosyl transferase activity and altered testosterone metabolism in these cells.

Kyle, E. *et al. Proc Ann Meet Am Assoc Cancer Res* (1995) 36:A2310 showed that genistein enhances cellular adhesion of prostate cancer cells but concluded that its growth inhibitory effects were independent of this. In contrast, Bergan, R.C. *et al.* (the same research group) *ibid.* A325, postulated that genistein could prevent prostate cancer cell transition from localized disease to systemic because of its effect on cell adhesion.

In addition, the growth of cell lines derived from small cell lung cancer, promyelocytic leukemia, colon cancer and endothelial cells is inhibited by isoflavonoids. Tallett, A. *et al. Cancer Res* (1996) 18:4255-4263; Szende, B. *et al. Cell Biol Int* (1995) 19:903-911; Cockerill, G. *et al. Int Rev Cytol* (1995) 159:113-160. Endothelial cells are particularly important targets for the therapy of solid tumors because the growth of these cells is required for angiogenesis which, in turn, is essential for tumor progression.

There are also reports of a correlation between isoflavonoids and the inhibition of the growth of breast cancer cell lines. Several studies have recently shown that genistein inhibits proliferation of normal mammary epithelial cells and several breast cancer cell lines *in vitro*, including MCF-7, MCF-7-D40, T47D (estrogen receptor positive) and MDA-468 and SKBR3 (estrogen receptor negative). Peterson, G. *et al. Carcinogenesis* (1996) 17:1861; Peterson, G. *et al. Cell Growth and Differentiation* (1996) 7:1345; Hofmann, R. *Biochem. Biophys. Res. Commun.* (1995) 211:600; Peterson, G. *et al. Biochem. Biophys. Res. Commun.* (1991) 179:661.

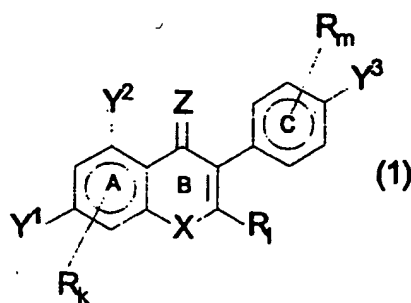
Thus, the art has attempted to evaluate the possible effect of soy products in general, and isoflavonoids occurring in soy in particular, on prostate cancer and breast

cancer. However, the results have been inconsistent as shown, in particular, by the studies of Naik (*supra*) and of Joseph (*supra*). The present invention relates to the modulation of the growth and proliferation of cells and tissues where the growth or proliferation is associated with regulation by sex hormones, by isoflavinoid-related compounds. Conditions associated with such regulation include, for example, prostate cancer, benign prostate hypertrophy (BPH), breast cancer and androgenic alopecia.

### Disclosure of the Invention

A number of conditions in mammalian subjects are subject at some level to regulation by the steroid sex hormones, such as estradiol, estrone, testosterone or other androgens and estrogens. These conditions include disorders associated with breast, ovary and prostate as well as conditions that seem less directly associated with sex organs such as androgenic alopecia. This invention relates to the discovery that these conditions are susceptible to treatment with a class of isoflavinoid-type compounds.

Accordingly, in one aspect, the invention is directed to a method to modulate the growth and/or proliferation of tissue or cells wherein said growth or proliferation is associated with regulation by the sex hormones, which method comprises contacting said tissue or cells with an amount of a compound of the formula



including forms thereof coupled to a targeting agent,  
wherein each R is independently a noninterfering substituent;

k is 0, 1 or 2;

l is 0 or 1;

m is 0, 1, 2, 3, or 4;

each Y is independently an electronegative electron-withdrawing polar group, wherein no more than two of Y<sup>1</sup>, Y<sup>2</sup> and Y<sup>3</sup> may optionally be H or R;

Z is =O, =S, =NH, =NR, <HH, <HR, or <RR; and

5 X is -O-, -S-, -NH-, -NR-, -CH<sub>2</sub>-, -CHR-, or -CR<sub>2</sub>-.

In another aspect, the invention is directed to pharmaceutical compositions containing a compound of formula 1, including forms coupled to a targeting agent for treating undesired conditions associated with sex hormone regulation.

10 In another aspect, the invention is directed to methods to treat undesired conditions associated with sex hormone regulation. These conditions include prostate cancer or benign prostate hypertrophy, malignancies of the breast or ovary, and androgenic alopecia. The method comprises contacting the appropriate cells or tissue with an amount of a compound of formula 1 including forms coupled to a targeting agent effective to alleviate the condition.

15

#### Brief Description of the Drawings

Figure 1 shows a graph of the growth inhibition by genistein on prostate tissue in histoculture.

20 Figure 2 is a graph showing the dose dependence of the inhibitory effect of genistein on prostate tissue in histoculture.

#### Modes of Carrying Out the Invention

25 The compounds of the invention may be used to modulate the growth of any cell or tissue whose growth is associated with regulation by sex hormones and either ultimately inhibited or stimulated thereby. Thus, the compounds of the invention are useful in the treatment of prostatic malignancies, benign prostatic hypertrophy (BPH), breast or ovarian cancer and androgenic alopecia.

30 As used herein, "treating" is defined as exerting a prophylactic or therapeutic effect. The prophylactic or therapeutic effect will ultimately modulate cell growth or proliferation. By "modulate" is meant a change in growth or proliferation; the change may be one of inhibition as in the case of tumor cells, or may be one of stimulation as

in the case of hair growth. Thus, the compounds of the invention are useful in both prevention and therapy of conditions associated with sex hormone regulation.

By "associated with sex hormone regulation" is meant that the condition responds to altered levels or nature of steroid sex hormones in a subject. These hormones include, for example, estrone, estradiol, and testosterone. Accordingly, the compounds of formula 1 can be supplied either alone, or coupled to a targeting agent.

"Targeting agent" is meant to include any ligand which selectively binds to the membrane of one or more cells or cell types. Methods of targeting drugs to a particular cell or tissue are well known in the cytotoxin art. In addition, Ouchi, T. *et al. Prog Polym Sci* (1995) 20:211-257, provides an excellent review of targeted drug delivery systems. Targeting agents can include autocrine, paracrine, endocrine and synaptic signaling molecules, antibodies, drugs and derivatives thereof. Preferred targeting agents of the invention are steroid hormones and derivatives thereof. Most preferred targeting residues are testosterone, dihydroxytestosterone, estrogen and derivatives thereof.

Steroid-based targeting agents are particularly useful in delivering the compounds of the invention in view of the regulation of the conditions to be treated by the steroid sex hormones. While not intending to be bound by any theory, applicants believe that the effectiveness of these targeting agents results from the mechanism by which steroid sex hormones are directed to particular cellular targets. In mammals, androgens and estrogens are associated with sex hormone binding globulin (SHBG) in the plasma. Petra, P.H. *et al. J Steroid Biochem* (1986) 24:45-49. The steroid hormone-globulin complexes interact specifically with membrane protein receptors on cells in the target tissue which reside next to free steroid-specific receptors that actively transport the steroid hormone into the cell. Only after such transport (specific to steroids) does the hormone interact with intracellular steroid receptors. Thus, the SHBG operates as a shuttle. The binding of the SHBG to prostatic cell membranes, for example, has been shown by Hryb, D.H. *Biochem Biophys Res Com* (1985) 128:432-440. Thus, useful targeting agents which are included in the conjugated forms of the compounds of formula 1 coupled to a targeting agent would employ the essential male or female sex hormones or analogs

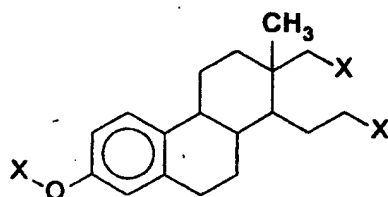


thereof with similar binding characteristics. Testosterone, dihydrotestosterone and estradiol can readily be bound through a linker to the 3- or 3- $\beta$  position of estradiol or of androst-4-ene-3 $\beta$ ,17 $\beta$ -diol, preferably through an ester bond. The 17 $\beta$ -OH group is preferably left free in order to bind to a lysine residue in the active pocket of SHBG.

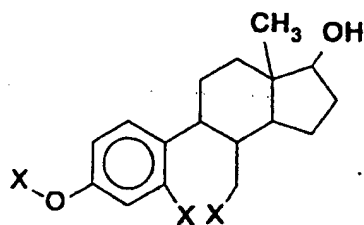
While the sex hormones themselves may be used as targeting residues, it may be desirable to use a derivative either with or without androgenic or estrogenic activity, or a derivative which is an antagonist of such activities. The latter class may be especially useful in treating hormone-dependent prostate or breast cancers.

Thus, suitable targeting agents that have antiandrogenic or antiestrogenic activity include 16,17-seco derivatives of the androstane and estrane series and the 6- and 7-derivatives of estrone and equilenin.

Suitable steroid targeting agents include cyproterone, 16,17-seco estrane derivatives of the formula:

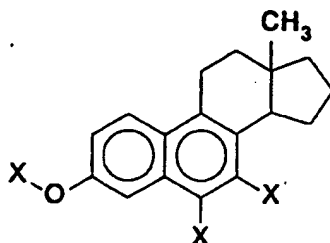


wherein X represents a functional group or an additional substituent; or 6,7-seco estrane derivatives of the formula:



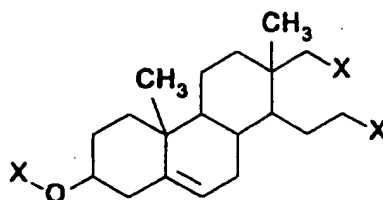
; or

6 or 7 derivatives of equilenin of the formula:



; or

16,17-seco androstane derivatives of the formula:



The targeting agent can be coupled to the compounds of formula 1 using standard linker technology. Suitable bifunctional linkers are available from Pierce Chemical Company, Rockford, IL. Advantageously included in the linker is a cleavable structure subject to degradation intracellularly so that the two components of the conjugate can act independently once inside the cell. Additional components may be inserted into the linker to confer water-solubility. For example, ionizable groups may be included in the linker, or a side chain may contain macromolecules such as poly-ethylene glycol (PEG).

The "conjugate forms" of formula 1, therefore, include the formula shown coupled to a targeting agent, optionally through a linker. The compound of formula 1 offers a number of functional groups that can serve as points of attachment for suitable linkers or targeting agents. Thus, any hydroxyl represented by Y could be thus used; similarly, functional groups contained in substituents represented by R can also be used.

As noted above, the compounds of formula 1 optionally contain noninterfering substituents in the A, B, and/or C rings.

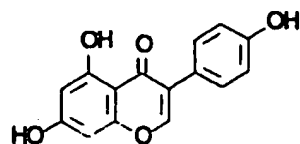
The noninterfering substituent designated by R may include  $-OR^1$ ;  $-NR^1$ ;  $-NO_2$ ;  $-SR^1$ ;  $-SOR^1$ ;  $-SO_2R^1$ ;  $-SO_3R^1$ ; halo, including  $-F$ ,  $-Cl$ ,  $-Br$  and  $-I$ ;  $-CR^1O$ ,  $-COOR^1$ ;  $-OOCR^1$ ,  $-CONR^1$ ,  $-NR^1COR^1$ ,  $-CN$ , and  $-CF_3$ , wherein  $R^1$  is defined as H

or alkyl 1-6C. R may also be a substituted or unsubstituted hydrocarbon radical optionally containing one or more heteroatoms. Hydrocarbon radicals include straight chain, branched chain or cyclic moieties, optionally containing one or more  $\pi$ -bonds. Thus, included are straight chain alkyls such as ethyl, butyl, octyl, dodecyl, and the like, branched chain forms of these alkyl substituents, radicals containing cyclic moieties such as 2-cyclohexylethyl, straight chain alkenyls such as 2-butenyl and its branched chain forms, and those containing cyclic moieties, including aryl moieties. Thus, included within the hydrocarbon radicals are alkyl, alkenyl, alkynyl, aryl, arylalkyl, arylalkenyl, arylalkynyl, and corresponding radicals which contain one or more heteroatoms. For example, the aryl radical may be in the form of pyrimidyl or thiophenyl. The "hydrocarbon" radical may thus include, for example,  $\text{CH}_3\text{CH}_2\text{NCH}_2\text{CH}_2-$ ,  $\text{CH}_3\text{OCH}_2\text{CH}_2-$ , and the like. The hydrocarbon radical may also contain monovalent substitutions containing heteroatoms such as halo,  $-\text{NO}_2$ ,  $-\text{CN}$ ,  $-\text{SO}_3\text{R}^1$ , and the like.

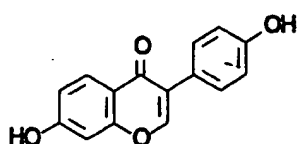
Preferred are hydrocarbon radicals containing 20C or less, preferably 15C or less, more preferably 10C or less, and still more preferably 6C or less. Especially preferred are methyl, ethyl, propyl, isopropyl, butyl, isobutyl and t-butyl. Particularly preferred embodiments of R are  $-\text{OR}^1$ ,  $-\text{NR}^1$ ,  $-\text{COOR}^1$  and alkyl (1-6C).

Preferred embodiments of the compound of formula 1 are those wherein Z is  $=\text{O}$  or is  $<\text{HH}$  and wherein each of  $\text{Y}^1$ ,  $\text{Y}^2$  and  $\text{Y}^3$  is independently H, OH or  $\text{NH}_2$ , but no more than two, preferably no more than one, Y is H. Also preferred are embodiments wherein X is  $-\text{O}-$ , or is  $-\text{NH}-$ . Also preferred are embodiments wherein k and m are 0 or 1 and wherein l is 0. More preferred are embodiments wherein all of k, l and m are 0.

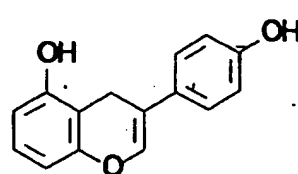
Particularly preferred embodiments of the compounds of formula 1 include those structures found as components of soy, including genistein, daidzein and equol. These have the formulas as shown below.



Genistein



Daidzein

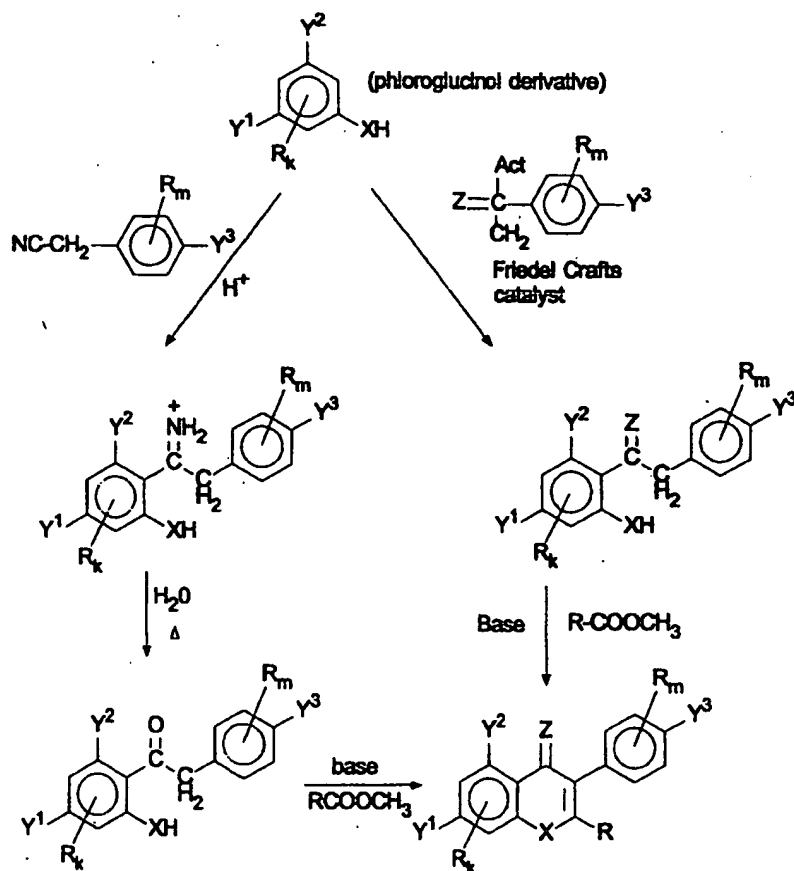


Equol

Other preferred compounds are 4'-deoxygenistein and 4'-aminogenistein.

The compounds of formula 1 may be prepared by a variety of methods known to those skilled in the art of organic chemistry. In addition, some of the compounds of formula 1 are commercially available or may be isolated from soy. Reaction scheme 1, below, illustrates methods which may be used to synthesize the compounds of formula 1.

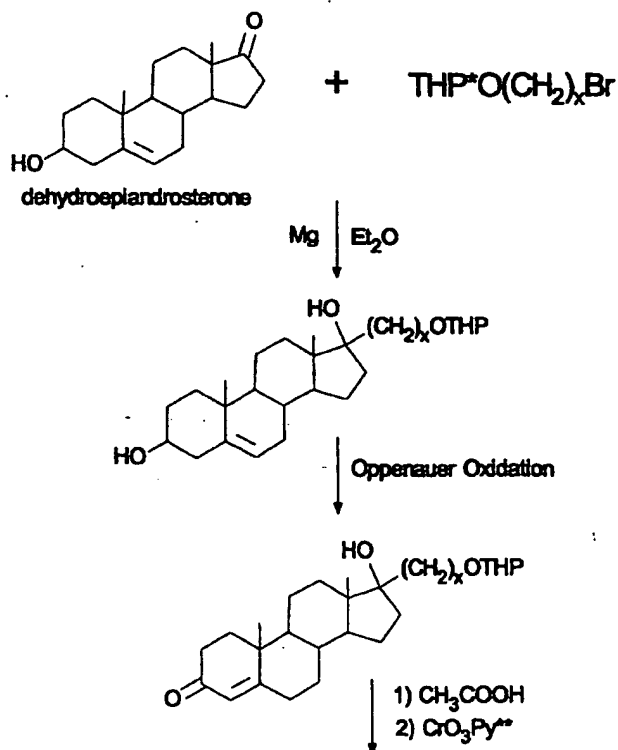
### Reaction Scheme 1



wherein R, X, Y, Z, k, l and m are as defined above and Act is a reactive group.

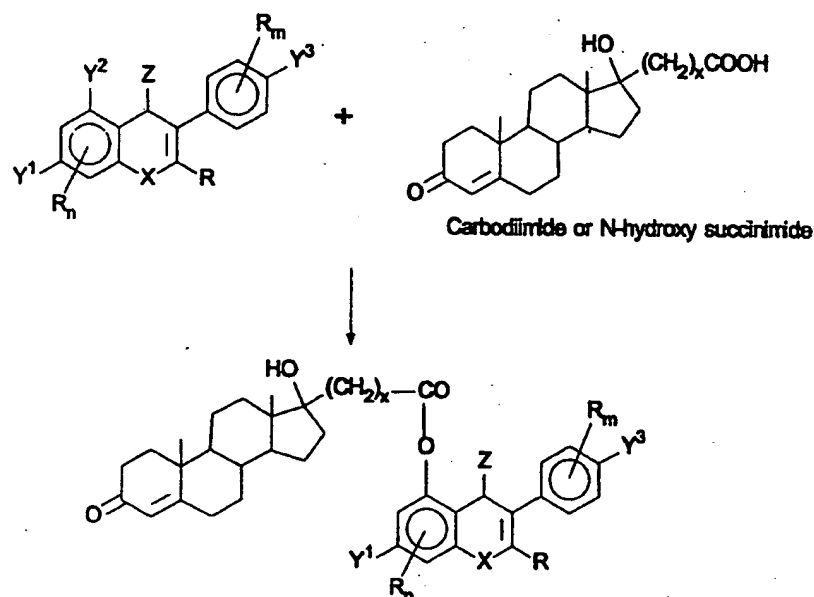
Methods for the preparation of the conjugated forms of compounds of formula 1 are shown in scheme 2 below. First, as shown in reaction scheme 2, a targeting residue (in this example, androsterone), is derivatized and coupled to a bifunctional linker, L, which may be substituted with moieties which give the conjugate form of formula 1 a desired characteristic. For example, if a compound of formula 1 is water insoluble, a water soluble derivative could be synthesized by including a protected amino group in the linker, and then deprotecting that amino group in the last step of conjugate synthesis to form the corresponding ammonium salt. In Scheme 2, below, the starting targeting residue, androsterone, is converted to an intermediate testosterone derivative, coupled to a linker and activated for further coupling.

Reaction Scheme 2



\*THP = tetrahydropyranyl

\*\*a mixture of  $(\text{PyH}^+)_2\text{CrO}_4$  and  $(\text{PyH}^+)_2\text{Mn}_2\text{O}_7$  in pyridine



## 5 Administration and Use

The compounds including the conjugates with targeting agent of the invention are administered at an effective dosage, i.e., that amount which, when administered to a mammal in need thereof, is sufficient to effect treatment. Administration of the active compounds can be via any of the accepted modes of administration for agents that serve similar utilities.

It will be noted that the compounds of the invention can be used individually or more than one compound falling within the scope of the invention can be used in admixture. In addition, other pharmaceutically active components can be included, such as analgesics, antibiotics, or other combination products.

The level of the drug in a formulation can vary within the full range employed by those skilled in the art, e.g., from about 0.01 percent by weight (w/w) to about 99.99% of the drug based on the total formulation and about 0.01% to 99.99% excipient. Preferably the drug is present at a level of about 10% to about 70%, the remainder being excipient or other drugs in combination.

Generally, an acceptable daily dose is about 0.1-500 mg/kg body weight of the recipient per day, preferably about 0.5-100 mg/kg body weight per day, and most preferably about 1-15 mg/kg body weight per day, depending upon the individual, the nature and severity of the prostate condition being treated. Such optimization is well within ordinary skill in the art.

Administration can be via any accepted systemic or local route, for example, via parenteral, oral, intravenous, nasal, bronchial inhalation (i.e. aerosol formulation), transdermal or topical routes, in the form of solid, semi-solid or liquid dosage forms, such as for example, tablets, suppositories, pills, capsules, powders, solutions, suspensions, aerosols, emulsions or the like, preferably in unit dosage forms suitable for simple administration of precise dosages. The compositions will include a conventional pharmaceutical carrier or excipient and an active compound of the invention and, in addition, may include other medicinal agents, pharmaceutical agents, carriers, adjuvants, etc. Carriers can be selected from the various oils, including those of petroleum, animal, vegetable or synthetic origin, for example, peanut oil, soybean oil, mineral oil, sesame oil, and the like. Water, saline, aqueous dextrose, and glycols are preferred liquid carriers, particularly for injectable solutions. Suitable pharmaceutical carriers include starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol, and the like. Other suitable pharmaceutical carriers and their formulations are described in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition.

If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, such as for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate, etc.

For intravenous injection the compound or compounds of the invention are dissolved in a suitable solvent or incorporated in a liposomal formulation followed by dispersal into an acceptable infusion fluid. A typical daily dose can be administered by one infusion, or by a series of infusions spaced over periodic intervals.

For oral administration, a pharmaceutically acceptable, nontoxic composition is formed by the incorporation of any of the normally employed excipients, such as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, gelatin, sucrose, magnesium carbonate, and the like. Thus, the composition will contain, along with the active ingredient, a diluent such as lactose, sucrose, dicalcium phosphate, and the like; a disintegrant such as starch or derivatives thereof; a lubricant such as magnesium stearate and the like; and a binder such as a starch, polyvinylpyrrolidone, gum acacia, gelatin, cellulose and derivatives thereof, and the like. Such compositions take the form of solutions, suspensions, tablets, pills, capsules, powders, sustained release formulations and the like. Such compositions may contain between 0.01% (w/w) and 99.99% of the invention compound, but preferably such compositions will contain between 25% and about 80%.

Pharmaceutical formulations based on liposomes have benefits related to favorable changes in tissue distribution and pharmacokinetic parameters that result from liposome entrapment of drugs, and may be applied to the compounds of the present invention by those skilled in the art. These formulations can be designed to either target drug to disease sites or the reticuloendothelial system, to increase duration of drug action or to divert a drug away from organs that are particularly sensitive to its toxic effects.

Controlled release liposomal liquid pharmaceutical formulations for injection or oral administration are described in U.S. Patent No. 4,016,100. Liposomal applications for oral drug delivery of a lyophilized liposome/peptide drug mixture filled into intestine capsules are described in U.S. Patent No. 4,348,384.

For systemic administration via suppository, traditional binders and carriers include, for example, polyalkaline glycol or triglycerides (e.g., PEG 1000 (96%) and PEG 4000 (4%)). Such suppositories may be formed from mixtures containing active ingredients in the range of wt/wt% from about 0.5% to about 10%; preferably from about 1% to about 2%.

Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, etc. an active compound (about 0.5% to about



20%), as described above, and optional pharmaceutical adjuvants in a carrier, such as for example, water, saline, aqueous dextrose, glycerol, ethanol and the like, to thereby form a solution or suspension.

The following examples are intended to illustrate but not to limit the invention.

#### Example 1

##### Effect of Genistein on Prostate Size in Mice

Two groups of five mice each, one group fed 100 mg/kg body wt/day of genistein and the other fed only excipient were compared with respect to body weight and prostate weight over the experimental period of 35 days. The results are shown in Table 1 where "G" represents mice administered genistein and "C" represents control mice.

| Table 1       |                                 |                               |                        |  |
|---------------|---------------------------------|-------------------------------|------------------------|--|
| Mouse # and R | Initial Body Weight 12/9/96 gms | Final Body Weight 1/13/97 gms | Prostate weight - mgms | Prostate weight - mgm/ body weight - gms |
| 1. G          | 37.1                            | 35.2                          | 12.4                   | 0.35                                     |
| 2. G          | 29.1                            | 29.9                          | 9.8                    | 0.33                                     |
| 3. G          | 29                              | 33.8                          | 8.1                    | 0.24                                     |
| 4. G          | 28.4                            | 34                            | 12.7                   | 0.37                                     |
| 5. G          | 27.5                            | 28.8                          | 9.3                    | 0.32                                     |
| $\bar{x}$     |                                 |                               | $\bar{x} = 10.46$      | $\bar{x} = 0.322$                        |
| 6. C          | 20.5                            | 26                            | 14                     | 0.52                                     |
| 7. C          | 38.5                            | 41.9                          | 14.7                   | 0.35                                     |
| 8. C          | 36.0                            | 40                            | 14.2                   | 0.35                                     |
| 9. C          | 33.4                            | 39                            | 9.7                    | 0.25                                     |
| 10. C         | 26.3                            | 27.2                          | 16                     | 0.59                                     |
| $\bar{x}$     |                                 |                               | $\bar{x} = 13.72$      | $\bar{x} = 0.414$                        |

As shown in the table, the control group had a mean prostate weight of 13.72 mg while the group fed genistein had a mean prostate weight of only 10.46 mg. The body weights in the two groups were comparable. Thus, the ratio of prostate weight in milligrams to body weight in grams was only 0.322 for the genistein-fed group and 0.414 for the controls. A paired t-test indicated a significant decrease ( $p=0.047$ ) in prostate weights in the genistein compared to control mice. This is the first demonstration of *in vivo* inhibition of prostate size by genistein.

### Example 2

#### Effect of Genistein on Prostate Cancer Tissue in Histoculture

Histocultures of prostate tissue exhibiting benign hypertrophy were evaluated as follows:

#### Methods

The source of prostatic tissues was either untreated surgical specimens from patients undergoing transurethral resection of the prostate (TURP) for BPH or radical prostatectomy specimens from patients undergoing surgery for early-stage prostate

cancer. In the latter instance, both BPH and prostate cancer tissue were available for some of the specimens.

Histoculture studies were done as previously reported using 2 ml wells in plastic culture plates containing 6 wells per plate. Geller, J. *et al. The Prostate* (1992) 21:269-278. A detailed description of tissue preparation and subsequent sponge-gel-matrix supported histoculture has been previously published. Geller, J. *et al. The Prostate* (1992) 21:269-278. An abbreviated description is as follows:

#### Tissue Preparation

Radical prostatectomy specimens from patients with early prostate cancer were placed in Minimal Essential Media (MEM) solution on ice in the operating room. Each specimen was weighed, measured and the surface was marked with green ink to aid the pathological staging of the tumor. The tissue was cut serially in its anatomical position, so that the 50% of the gland above and below a mid-line axial cut was labeled as anterior and posterior, respectively. A sagittal cut going from distal to proximal divided the prostate into right and left sections. Approximately 0.5 cm cuts were then made beginning at the distal urethral end of the prostate with each section identified with a letter label. Representative specimens of cancer and BPH were selected for histoculture. Anatomical mirror image sections of the specimens were kept for determination of microscopic anatomy. Tissues obtained from TURP were carefully dissected to remove any discolored damaged tissue from the individual chips. The tissues were placed in ice-cold MEM solution prior to histoculture.

#### Histoculture

BPH and cancer specimens, whether obtained from radical prostatectomy specimens or from TURP specimens, were separately cut into 1 cubic mm minces. The tissue cubes were planted on Gelfoam collagen-gel matrices with media containing MEM and appropriate concentrations of dihydroxytestosterone (DHT) and genistein in tissue culture wells with 2 ml media.

DHT treated histocultures were used as the untreated control and represented maximal stimulation of <sup>3</sup>H-thymidine incorporation into prostatic tissue. The percent

reduction in  $^3\text{H}$ -thymidine incorporation/ $\mu\text{g}$  protein in the presence of varying concentrations of genistein was calculated as a percent decrease in  $^3\text{H}$ -thymidine incorporation/ $\mu\text{g}$  protein from controls as a result of the biologic effects of genistein. Histoculture, as previously described (Geller, J. *et al. The Prostate* (1992) 21:269-278; Geller, J. *et al. The Prostate* (1994) 25:206-209; Geller, J. *et al. The Prostate*, In Press), was performed for five days. DHT ( $2 \times 10^{-6}\text{M}$ ) was added daily on days 2-5 of histoculture to all tissues. Genistein in concentrations ranging from 1.25  $\mu\text{g}/\text{ml}$  (4.6  $\mu\text{M}$ ) up to 15  $\mu\text{g}/\text{ml}$  (55.3  $\mu\text{M}$ ) was added on day-2 to BPH and cancer histocultures (Figure 1).

DMSO, which was the diluent for the genistein, was added in similar amounts to all cultures. Ethanol, which was the diluent for DHT, was added in equal amounts to all other cultures.  $^3\text{H}$ -thymidine was added on day 5 to all cultures. Incubations were stopped 24 hours later. The tissues were kept frozen following incubation until analysis.

#### Measurement of $^3\text{H}$ -thymidine Incorporation

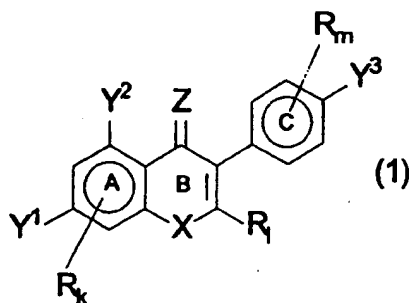
Processing of tissues for measurement of  $^3\text{H}$ -thymidine incorporation per  $\mu\text{g}$  of protein was done by first washing tissues to remove unbound  $^3\text{H}$ -thymidine. Tissues were then homogenized in sucrose buffer and centrifuged. The pellet obtained was digested with benzomethonium hydroxide for measuring  $^3\text{H}$ -thymidine counts per minute and the supernate was analyzed for protein concentration. Results were expressed as  $^3\text{H}$ -thymidine incorporation per  $\mu\text{g}$  protein for both BPH and cancer tissues histocultured with DHT, HF, and genistein, alone or in combination.

The results are shown in Figure 1 and Figure 2. In a single experiment, as shown in Figure 1, as little as approximately 5  $\mu\text{M}$  genistein showed 50% inhibition of a mixture of BPH and prostate cancer tissue growth in histoculture, compared to the dihydrotestosterone (DHT) control. 100% inhibition of growth was achieved at 10  $\mu\text{g}/\text{ml}$  of genistein. The average dose response curve for BPH tissue treated with genistein in histoculture (based on 10 experiments) and measured by the percent decrease in incorporated thymidine/ $\mu\text{g}$  of protein is shown in Figure 2. A 30% decrease in incorporation was shown at very low concentrations of 1.25  $\mu\text{g}/\text{ml}$ ; at

15 µg/ml over 60% inhibition was shown. Average values for genistein inhibition of prostate cancer growth are similar to those shown in Figure 2.

Claims

1. A method to modulate the growth and/or proliferation of tissue or cells wherein said growth or proliferation is associated with regulation by the sex hormones, which method comprises contacting said tissue or cells with an amount of a compound of the formula



- including forms thereof coupled to a targeting agent, wherein each R is independently a noninterfering substituent;
- 10 k is 0, 1 or 2;  
l is 0 or 1;  
m is 0, 1, 2, 3, or 4;  
each Y is independently an electronegative polar group, wherein two, but only two of Y<sup>1</sup>, Y<sup>2</sup> and Y<sup>3</sup> may optionally be H or R;
- 15 Z is =O, =S, =NH, =NR, <HH, <HR, or <RR; and  
X is -O-, -S-, -NH-, -NR-, -CH<sub>2</sub>-, -CHR-, or -CR<sub>2</sub>-.

2. The method of claim 1 wherein Z is =O, =S, =NH, =NR<sup>1</sup>, <HH, <HR<sup>1</sup> or <R<sup>1</sup>R<sup>1</sup>, wherein R<sup>1</sup> is H or alkyl (1-6C); and/or
- 20 wherein X is -O-, -S-, -NH-, -NR<sup>1</sup>-, -CH<sub>2</sub>-, -CHR<sup>1</sup>-, or -CR<sup>1</sup><sub>2</sub>- wherein R<sup>1</sup> is H or alkyl (1-6C); and/or
- wherein each R is independently alkyl (1-6C), halo, -COOR<sup>1</sup>, -CN, -CF<sub>3</sub>, -OR<sup>1</sup>, or -NR<sup>1</sup>, wherein R<sup>1</sup> is H or alkyl (1-6C); and/or
- wherein Z is <H<sub>2</sub> or =O and X is O; and/or

wherein k, l, and m are 0; and/or

wherein each Y is independently -H, -OH, or -NH<sub>2</sub>.

5           3.     The method of claim 1 or 2 wherein each Y is independently -H, -OH  
or -NH<sub>2</sub>, and only one Y is H.

          4.     The method of claim 1 wherein the compound of formula 1 is selected  
from the group consisting of genistein, daidzein, equol, 4'-deoxygenistein and  
4'-aminogenistein.

10

          5.     The method of any of claims 1-4 wherein the compound of formula 1  
is coupled, optionally through a linker, to a targeting agent.

          6.     The method of claim 5 wherein said targeting agent is a steroid sex  
15 hormone or derivative thereof.

          7.     The method of claim 6 wherein said hormone is testosterone,  
dihydroxytestosterone, estradiol, estrone or a derivative thereof.

20           8.     The method of claim 1 wherein said tissue is abnormal prostate tissue,  
or

wherein said cells are prostate cancer cells, or

wherein said cells are breast cancer cells, or

wherein said cells are hair follicle cells of the scalp.

25

          9.     A pharmaceutical composition for use in a method to modulate the  
growth and/or proliferation of tissue or cells wherein said growth or proliferation is  
associated with regulation by the sex hormones, which method comprises contacting  
said tissue or cells with an amount of a composition comprising an amount of the  
30 compound of claim 1 or a form thereof conjugated to a targeting agent effective to

prevent or inhibit the growth and/or proliferation of said tissue or cells in combination with at least one pharmaceutically acceptable excipient and in unit dosage form.

10. The composition of claim 10 which further contains an additional  
5 active ingredient.



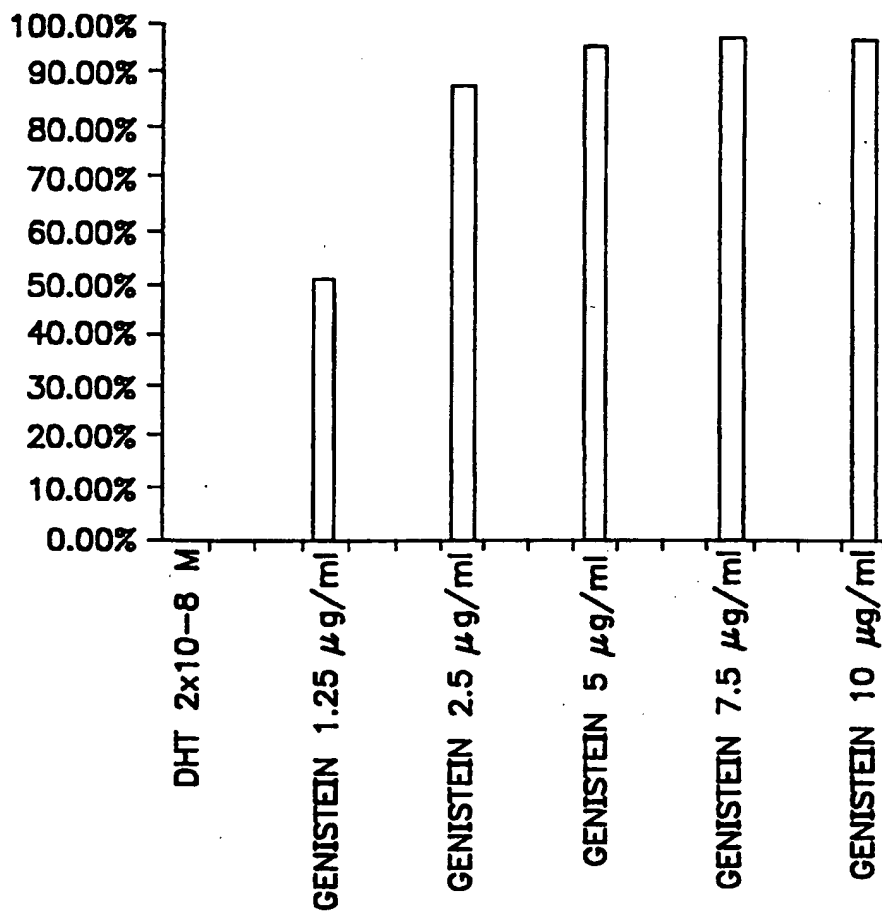


FIG. 1

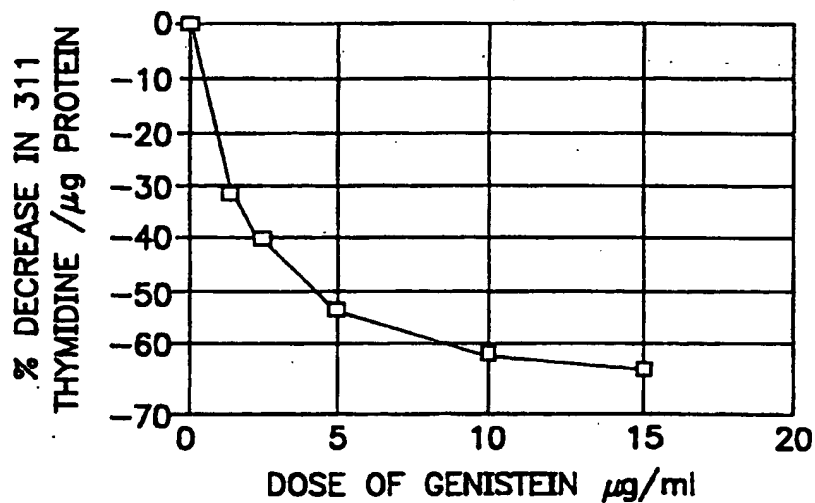


FIG. 2

# INTERNATIONAL SEARCH REPORT

Intern. Application No.

PCT/US 98/08484

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K31/35

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. |
|------------|--|-----------------------|
| P,X        | J. GELLER ET AL.: "GENISTEIN INHIBITS THE GROWTH OF HUMAN-PATIENT BPH AND PROSTATE CANCER IN HISTOCULTURE"<br>PROSTATE,<br>vol. 34, no. 2, February 1998, pages 75-79, XP002077078<br>see the whole document | 1-4,8-10              |
| P,X        | WO 97 29779 A (UNIV MINNESOTA)<br>21 August 1997<br>see abstract; claims; examples<br>-/-  | 1-5,8-10              |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

10 September 1998

Date of mailing of the international search report

23/09/1998

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Hoff, P

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/08484

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. |
|----------|--|-----------------------|
| P,X      | WO 98 08503 A (KELLY GRAHAM EDMUND<br>;JOANNOU GEORGE EUSTACE (AU); NOVOGEN<br>RESEARCH) 5 March 1998<br>see abstract<br>see page 9, line 8 - line 10<br>see page 31, line 4 - line 17; claims;<br>examples 1,8-11   | 1-4,8-10              |
| X        | WO 93 23069 A (KELLY GRAHAM EDMUND)<br>25 November 1993<br>see abstract<br>see page 8, paragraph 2 - page 9, last<br>paragraph<br>see page 15, last paragraph - page 16,<br>last paragraph; claims   | 1-4,8-10              |
| X        | PATENT ABSTRACTS OF JAPAN<br>vol. 013, no. 308 (C-617), 14 July 1989<br>& JP 01 096126 A (KAO CORP),<br>14 April 1989<br>see abstract<br>& DATABASE WPI<br>Week 8921<br>Derwent Publications Ltd., London, GB;<br>AN 89-155044<br>& JP 01 096126 A (KAO CORP), 14 April 1989<br>see abstract   | 1-4,8-10              |
| X        | DATABASE WPI<br>Section Ch, Week 8921<br>Derwent Publications Ltd., London, GB;<br>Class B02, AN 89-155042<br>XP002077086<br>& JP 01 096124 A (FIJI REBIO KK)<br>, 14 April 1989<br>see abstract<br>& CHEMICAL ABSTRACTS, vol. 111, no. 21,<br>20 November 1989<br>Columbus, Ohio, US;<br>abstract no. 187601, "ANTICANCER AGENTS<br>CONTAINING FLAVONOIDS"<br>see abstract<br>& JP 01 096124 A (FIJI REBIO KK)<br>14 April 1989 | 1-4,8-10              |
| X        | WO 96 39832 A (KUNG PATRICK C)<br>19 December 1996<br>see abstract<br>see page 6, line 6 - page 7, line 35<br>see claims 1-10; example 4   | 1-4,8-10              |
| X        | DE 44 32 947 A (NEW STANDARD GMBH)<br>21 March 1996<br>see the whole document  | 1-4,8-10              |

-/-

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/08484

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. _____ |
|------------|--|-----------------------------|
| X          | <p>WO 96 06116 A (UNIV MINNESOTA)<br/> 29 February 1996<br/> see abstract<br/> see page 12, line 21 - page 13, line 12<br/> see page 16, line 21 - line 27; claims</p>   | 1-5,8-10                    |
| X          | <p>Y. WANG ET AL.: "SOY ISOFLAVONES DECREASE<br/> THE HIGH-FAT GROWTH OF HUMAN PROSTATE<br/> CANCER: RESULTS OF A IN VITRO AND ANIMAL<br/> STUDIES"<br/> THE JOURNAL OF UROLOGY,<br/> vol. 153, no. S4, 1995, page 269A<br/> XP002077079<br/> see the whole document</p>                               | 1-4,8-10                    |
| X          | <p>M.C. BOSLAND ET AL.: "INHIBITION OF HUMAN<br/> PROSTATE CANCER CELL PROLIFERATION BY<br/> GENISTEIN"<br/> PROCEEDINGS OF THE AMERICAN ASSOCIATION<br/> FOR CANCER RESEARCH,<br/> vol. 38, March 1997, page 262 XP002077080<br/> see the whole document</p>  | 1-4,8-10                    |
| X          | <p>J.F. SANTIBANEZ ET AL.: "GENISTEIN<br/> INHIBITS PROLIFERATION AND IN VITRO<br/> INVASIVE POTENTIAL OF HUMAN PROSTATIC<br/> CANCER CELL LINES"<br/> ANTICANCER RESEARCH,<br/> vol. 17, no. 2A, March 1997, pages<br/> 1199-1204, XP002077081<br/> see the whole document</p>                        | 1-4,8-10                    |
| X          | <p>S. BARNES ET AL.: "POTENTIAL ROLE OF<br/> DIETARY ISOFLAVONES IN THE PREVENTION OF<br/> CANCER"<br/> ADVANCES IN EXPERIMENTAL MEDICINE AND<br/> BIOLOGY,<br/> vol. 354, 1994, pages 135-147, XP002077082<br/> see the whole document</p>  | 1-4,8-10                    |
| X          | <p>G. PETERSON ET AL.: "GENISTEIN INHIBITS<br/> BOTH ESTROGEN AND GROWTH FACTOR-STIMULATED<br/> PROLIFERATION OF HUMAN BREAST CANCER"<br/> CELL GROWTH AND DIFFERENTIATION,<br/> vol. 7, no. 10, 1996, pages 1345-1351,<br/> XP002077083<br/> cited in the application<br/> see the whole document</p> | 1-4,8-10                    |
| -/-        |  |                             |

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/08484

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. _ |
|------------|---|-------------------------|
| X          | A.I. CONSTANTINOU ET AL.: "INHIBITION OF N-METHYL-N-NITROSOUREA-INDUCED MAMMARY TUMORS IN RATS BY THE SOYBEAN ISOFLAVONES" ANTICANCER RESEARCH, vol. 16, no. 6A, 1996, pages 3293-3298, XP002077084<br>see the whole document | 1-4,8-10                |
| A          | DATABASE WPI<br>Section Ch, Week 8650<br>Derwent Publications Ltd., London, GB;<br>Class B02, AN 86-329435<br>XP002077087<br>& JP 61 246124 A (YAMANOUCHI PHARM CO LTD)<br>, 1 November 1986<br>see abstract                  | 1-10                    |

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/08484

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claim(s) 1-8  
is(are) directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☒ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such  
an extent that no meaningful International Search can be carried out, specifically:  
See FURTHER INFORMATION sheet PCT/ISAY/210
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

In view of the large number of compounds which are defined by the general formula of claim 1 the search was limited to the inventive part of the molecule and to the compounds mentioned in claim 4 (Art.6.PCT; Guidelines Part B, Chapt.II.7 last sentence and Chapt.III,3.7).

Claims searched completely 4

Claims searched incompletely 1-3, 5-10

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. Appl. No.

PCT/US 98/08484

| Patent document<br>cited in search report |   | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
|---|---|---------------------|----------------------------|---------------------|
| WO 9729779                                | A | 21-08-1997          | AU 1958897 A               | 02-09-1997          |
| WO 9808503                                | A | 05-03-1998          | AU 4003497 A               | 19-03-1998          |
| WO 9323069                                | A | 25-11-1993          | AU 683838 B                | 27-11-1997          |
|   |   |                     | AU 4052593 A               | 13-12-1993          |
|   |   |                     | EP 0656786 A               | 14-06-1995          |
|   |   |                     | JP 7506822 T               | 27-07-1995          |
|   |   |                     | NO 944435 A                | 18-11-1994          |
|   |   |                     | NZ 252051 A                | 28-10-1996          |
| WO 9639832                                | A | 19-12-1996          | US 5639785 A               | 17-06-1997          |
|   |   |                     | AU 5970496 A               | 30-12-1996          |
| DE 4432947                                | A | 21-03-1996          | NONE                       |                     |
| WO 9606116                                | A | 29-02-1996          | US 5587459 A               | 24-12-1996          |
|   |   |                     | AU 3216895 A               | 14-03-1996          |
|   |   |                     | EP 0776338 A               | 04-06-1997          |
|   |   |                     | JP 10505056 T              | 19-05-1998          |